DAY-LENGTH AND TERMINATION OF PHOTOREFRACTORINESS IN THE ANNUAL TESTICULAR CYCLE OF THE TRANSEQUATORIAL MIGRANT DOLICHONYX (THE BOBOLINK) [1]

WILLIAM L. ENGELS

Department of Zoology, University of North Carolina, Chapel Hill, N. C.

At the end of a reproductive season the testes of birds undergo a regression, which results in minute, inactive gonads composed of small tubules formed almost entirely of spermatogonia, the tubules separated by masses of undifferentiated interstitial tissue. In those passeriform birds in which testicular recrudescence is under photoperiodic control, periods of long day-length fail not only to prevent the occurrence of this regression but fail to stimulate recrudescence after the regression is completed. Hence, this period of the annual cycle is known as the *photorefractory phase* (the "preparatory phase" of Wolfson, 1958, p. 372). After some weeks of exposure to short photoperiods this refractoriness disappears; thereafter, long photoperiods again stimulate the mechanism which produces testicular recrudescence.

Among transequatorial migrants the existence of a photoperiodic mechanism, including a refractory period, has so far been demonstrated only in the bobolink, *Dolichonyx oryzivorus* (Engels, 1959, 1961; Wolfson and Westerhoff, 1960). It has been shown (Engels, 1961) that (1) exposed to the natural day-lengths of the northern hemisphere, as experienced by such temperate zone migrants as *Junco hyemalis* and *Zonotrichia albicollis,* the testicular cycle of *Dolichonyx* develops ultimately (April) approximately in normal phase; (2) *Dolichonyx* is able to overcome naturally induced, autumnal refractoriness on longer photoperiods (12 hours) than can at least some populations of *Junco* and *Zonotrichia;* but (3) the rate of response to long photoperiods (14 hours) following termination of refractoriness is slower in *Dolichonyx* than in the other two forms. However, as was pointed out (Engels, 1961, p. 146), the photoperiods used in these earlier experiments to release refractoriness were considerably shorter, and the duration of treatment considerably longer, than birds could be expected to experience in nature in a migration from the northern to the southern hemisphere soon after the September equinox.

The experiments now to be reported upon were designed (1) to compare *Dolichonyx* to north temperate zone migrants with respect to the timing of termination of refractoriness when exposed to the natural day-lengths of the north temperate zone, and (2) to determine the capacity of *Dolichonyx* to overcome refractoriness when exposed to photoperiods more nearly comparable to those normally experienced in post-nuptial transequatorial migration. Since only meager and scattered information on the timing of the southward transequatorial passage of *Dolichonyx* can be found in the literature, special effort was made to establish the

pertinent facts. The details which are presented below on autumnal migration in South America were obtained mostly from specimens in the collections of major museums in the United States.

MATERIALS AND METHODS

Twenty-one adult male bobolinks (*Dolichonyx oryzivorus*), all of which had experienced the natural day-lengths of the northern hemisphere during the preceding summer, were used in the experiments. Two were captured near Wilmington, North Carolina, in September, a few weeks before experimental treatment was begun ("autumn captures"); fifteen were captured near Gainesville, Florida,[2] in early May of the year of experimental treatment ("spring captures"); four had been in captivity one to two years ("second-year experimentals").

The birds were kept in an outdoor aviary, exposed to the natural day-lengths of Chapel Hill (Lat. 36° N.), until experimental illumination was begun at various times from October 2 to November 28. At the beginning of artificial lighting they were confined individually in small cages (each about 22 cm. × 25 cm. × 40 cm.) Each cage was furnished with a food hopper and two 100-cc. water-tubes. Food consisted of a mash formulated as a complete diet for egg-laying "game" birds; a small amount of soluble terramycin was added to the water. The lights used to provide the experimental photoperiods were automatically switched on and off by electrically operated time-switches. Eight different lighting schedules were used; details of the schedules, including light intensity, are given below. Light intensity was measured at perch-level.

The birds were examined weekly. Testicular recrudescence was determined by the development of black pigment in the beak, especially evident in the "mandible"; this pigmentation is caused directly by the male sex hormone (Engels, 1959). In seven bobolinks which were killed, during the winter of 1961–62, within a few days to a maximum of two weeks following the first appearance of this pigmentation, the testes averaged 179 mm.[3] per bird in volume (range, single testis, 32.5 mm.[3] to 131.5 mm.[3]) (previously unpublished data). The volume of an inactive testis, in males with light-colored beaks, is less than 2 mm.[3]

RESULTS

1. *Termination of refractoriness under natural day-lengths of Lat. 36° N.* (*Figure 1*)

Five groups of birds, two to four in each group, were used in this series of experiments. *Group A* (Group E of Engels, 1961, p. 143) consisted of two "autumn captures" removed from the aviary to an indoor, light-tight compartment on October 2 and exposed thereafter to constant daily 14-hour photoperiods (white fluorescent lights, intensity about 90 foot-candles.) Neither of these birds had developed beak pigmentation by late May, when observations were terminated.

Group B consisted of four birds, all "2nd year experimentals." They were exposed to natural day-lengths from late May until November 28, after which white
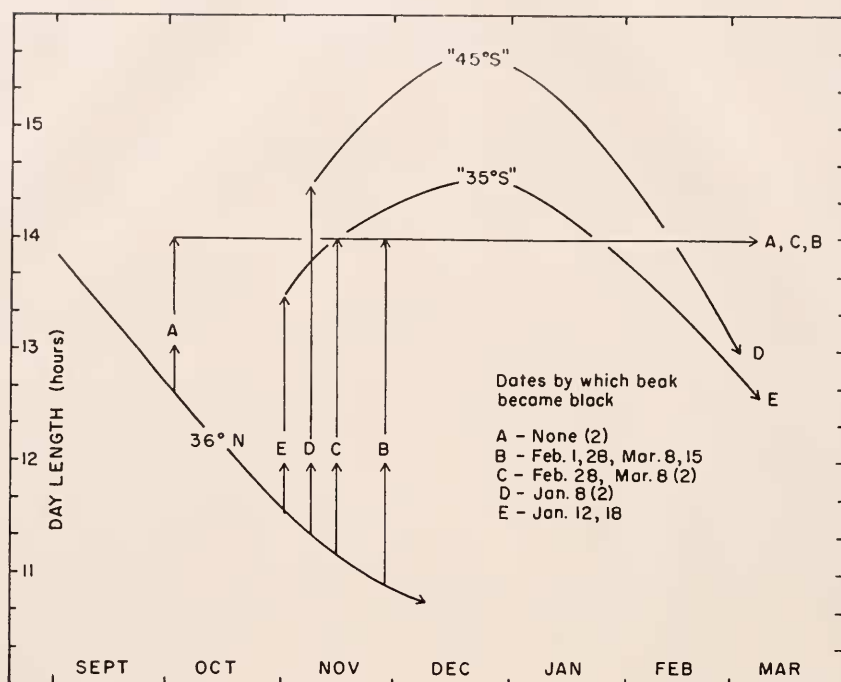
FIGURE 1. Experiments to determine the time of termination of photorefractoriness in bobolinks exposed to the natural day-lengths of Lat. 36° N. At various times between October 2 and November 28, day-length was abruptly increased either to 14 hours daily or to a simulation of the daily change in day-length (sunrise-sunset) occurring at that time in the southern hemisphere. For this treatment groups A and E were moved, at the times indicated, to light-tight compartments indoors; the other groups remained outdoors, where the artificial lighting was superimposed on the natural day-length. The development of black pigment in the beak is evidence of testicular recrudescence.

fluorescent lights (which insured a minimum intensity of 30 to 35 foot-candles) provided 14-hour daily photoperiods (5:15 AM–7:15 PM); these birds remained in the outdoor aviary, hence during the dark period were exposed to approximately normal light of the night sky. Beak pigmentation indicative of testicular recrudescence developed first in one bird during the week ending February 1, in the other three before March 15.

Group C consisted of three "spring captures"; they were subjected to exactly the same light schedules and other conditions as Group B except that the 14-hour photoperiods were begun almost two weeks earlier, on November 15. Beak pigmentation developed during the last week of February and first week of March.

Group D consisted of two "spring captures"; they were caged in an outdoor aviary where, beginning November 8, incandescent lamps (intensity about 45 foot-candles) provided photoperiods which approximated the changing sunrise-sunset day-lengths normally occurring during the months of November to March at Lat. 45° S. The abrupt change in photoperiod on the first day was from $11\frac{1}{4}$ hours to $14\frac{1}{2}$ hours; the photoperiod then increased gradually to more than $15\frac{1}{2}$ hours in

December. Both birds developed the nuptial pigmentation of the beak during the first week of January. (These lights were automatically switched on and off by an "astronomical-dial" time switch, geared to the daily changes in time of sunrise and of sunset at Lat. 45°, manufactured by the Sangamo Electric Co., Springfield, Illinois.)

Group E also consisted of two "spring captures." They were removed from an outdoor aviary on November 1 and thereafter subjected indoors to photoperiods corresponding to the changing sunrise-sunset day-lengths of Lat. 35° S. Beak pigmentation developed during the second and third weeks of January. (White fluorescent lights, intensity about 90 foot-candles, switched on and off by an "astronomical-dial" clock were used; between photoperiods the birds were in absolute darkness.)

General conclusion

The mechanism which stimulates testicular recrudescence in bobolinks is refractory to long photoperiods in early autumn, at least until October 1; when birds are held captive in the northern hemisphere, exposed to the natural day-lengths of Lat. 36° N., photorefractoriness is terminated sometime during October, definitely by November 1.

2. *The southward migration of bobolinks and the day-lengths experienced by them during migration (Figures 2, 3)*

Oberholser (1920) brought together data from a number of localities in the United States, gathered over various periods of years, which give for each locality an average "first date seen" and an average "last date seen," that is, average dates of arrival and departure. These data for the eastern United States in autumn are incorporated in the accompanying chart of latitudinal distribution (Fig. 2). Since 1947 "Audubon Field Notes," in an annual review of autumnal migration of North American birds, provides some additional data for points within the United States. Unfortunately, records chiefly only of an unusual nature (exceptionally early or exceptionally late observations) are published here, but there have been a few notices on peaks of abundance. All the "Audubon Field Notes" records, through 1960, are also represented in Figure 2. No such data are available for the migration south of the United States. Through the kind cooperation of a number of individuals and institutions I have been able to locate, in museums of this country, 89 specimens taken south of the United States and to compile the data on locality and date of collection.[3] Thirty-seven of these specimens were taken in September, October, and November. Together with the Oberholser and the "Audubon Field
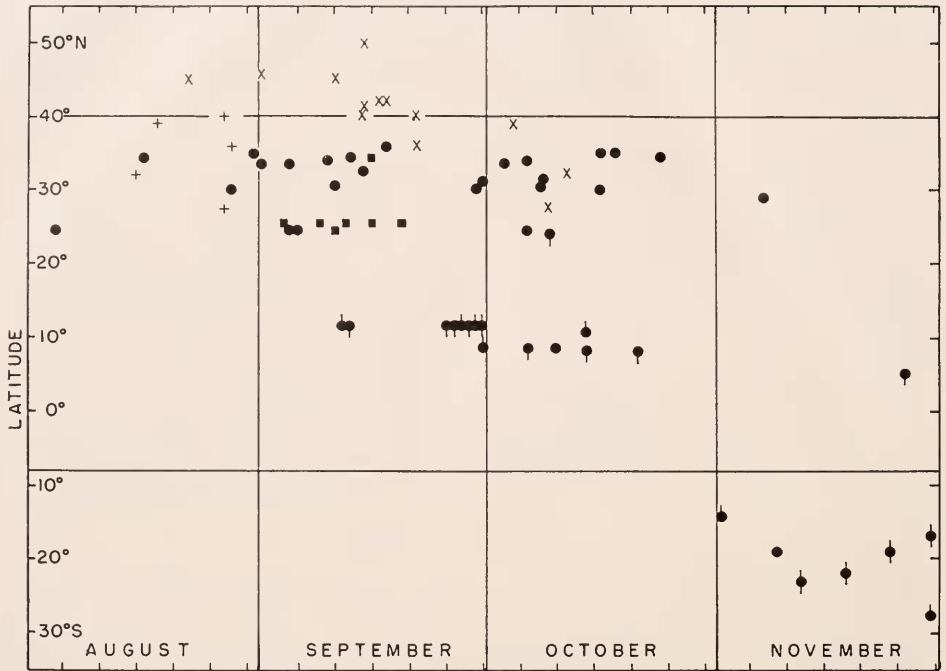
FIGURE 2. Latitudinal distribution of bobolinks from August through November. The horizontal lines approximate the southern border of the breeding grounds (Lat. 40° N.) and the northern border of the "wintering" grounds (Lat. 8° S.). Plain circles are from published sight records, mostly from *Audubon Field Notes* (vols. 1-14, 1947–1960) ; squares indicate "peaks of abundance" reported in the same journal; + and × indicate annual average dates of arrival and departure, respectively (average dates of "first seen" and "last seen"; data from Oberholser, 1920) ; circles with vertical lines indicate museum specimens (line above circle = male, below = female). The major part of the "wintering" grounds lie below about Lat. 14° S. (in Peru, Bolivia, Brazil [Matto Grosso], Paraguay and northern Argentina), but the species occurs regularly between November and March in the Trujillo and Lima districts of Peru, on the western slope of the Andes, at Lat. 8°–12° S. (Koepcke, 1961).

Notes" data, these records give a reasonably clear picture of the southward migration of bobolinks (Fig. 2). The breeding grounds, north of Lat. 40° N., are usually emptied by the end of the second or third week of September. Meanwhile, some individuals (which must have started their journey in August) have at that time already made the trans-Gulf or trans-Caribbean passage and are in Central or South America at Lat. 8°–11° N. Although some individuals may still be in the United States in late October, or exceptionally even in early November, some have reached the "wintering" area below Lat. 8° S. at least by November 1. In Figure 3 the same latitudinal distribution data are plotted against day-length (sunrise-sunset plus morning and evening civil twilight). The arrow drawn through this figure gives a rough approximation of the day-lengths experienced by an "average" bobolink during the southward transequatorial migration. Whatever the degree of validity of this approximation, there are certain limits on the day-length cycle which migrating bobolinks may experience, limits which are imposed
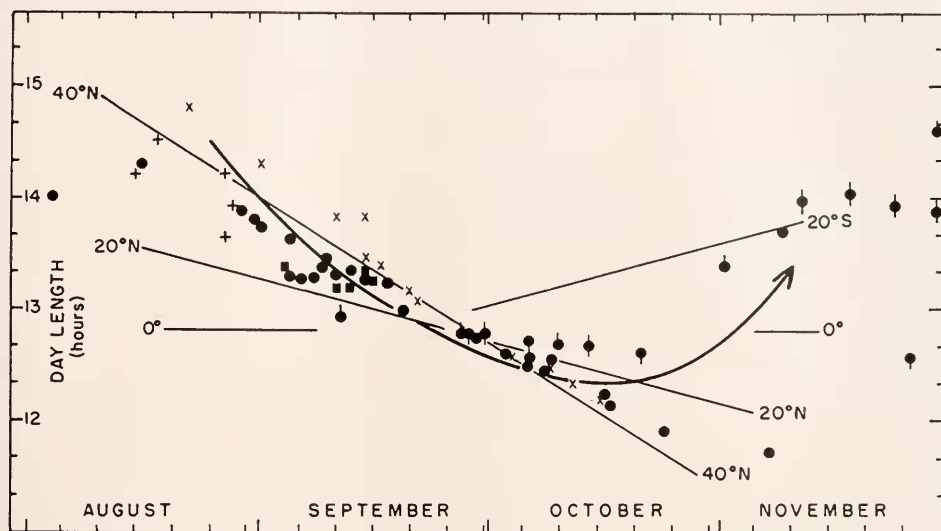
FIGURE 3. Day-lengths (including civil twilight) experienced by bobolinks from August through November. Same data and symbols as in Figure 2. The arrow roughly approximates the migration of an "average" bobolink. See text for discussion of limiting factors. (Day-length data from: Tables of Sunrise, Sunset and Twilight: Supplement to the American Ephemeris, 1946; U. S. Naval Observatory. Government Printing Office, Washington, D. C.)

by the changes in day-length at different latitudes, some of which also are shown in this figure. Any birds which might reach the equator during the third week of September would experience at about that time their shortest day-length, about 12 hours 45 ± 4 minutes (sunrise-sunset plus civil twilight); thereafter they would be exposed to gradually increasing day-lengths (until the December solstice). Any birds still north of the equator on October 1, at whatever latitude, would at that time also experience day-lengths of about 12 hours 45 ± 4 minutes. Day-lengths continue to decline in the northern hemisphere until the December solstice but at a progressively lower rate the lower the latitude. As a consequence of this phe-nomenon, and as is evident in Figure 3, a southwardly migrating bird begins to experience a progressive *increase* in day-length while still north of the equator in October, an even greater increase in November; it is again exposed to a little more than 12¾ hours of day-light when it reaches the equator. South of the equator, of course, day-lengths then everywhere are increasing above that level.

3. *Termination of refractoriness under day-lengths comparable to those experienced in transequatorial migration (Figure 4)*

In addition to the previously described Group A, three groups of bobolinks, three birds in each group, were used in this series of experiments. All of these nine birds were "spring captures" which had spent the summer in an outdoor aviary, exposed to the natural day-lengths of Lat. 36° N. As with Group A, they were brought indoors at the beginning of October into light-tight, ventilated compart-ments and exposed there to artificial photoperiods under white fluorescent lights. Between photoperiods they were in absolute darkness.
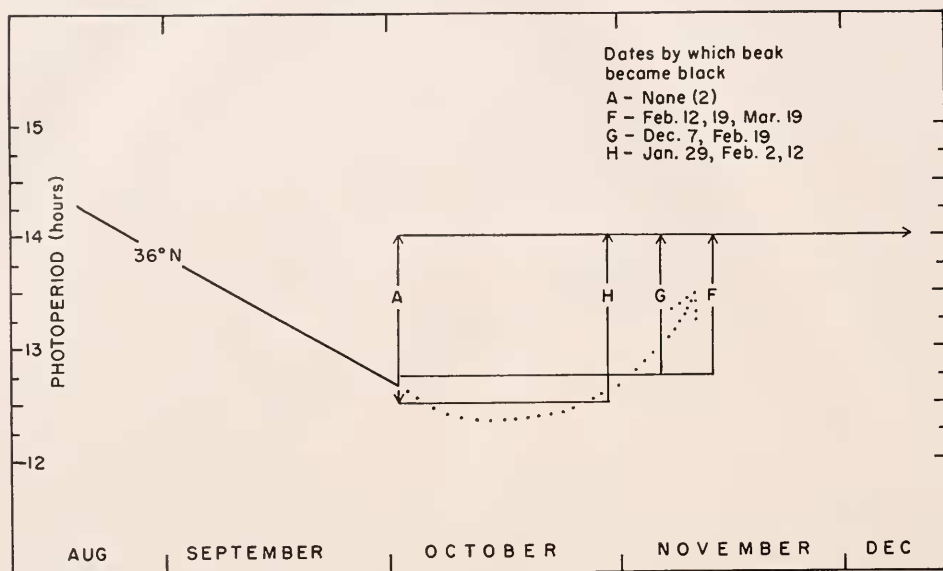
FIGURE 4. Experiments to determine length of photoperiod, and duration of treatment, which may release bobolinks from photorefractoriness during October and November. On October 2 ten birds were removed, from the natural day-lengths (including civil twilight) of Lat. 36° N., to constant daily photoperiods of different lengths, in four groups. Development of black pigment in the beak indicates testicular recrudescence. The dotted line is a rough approximation of day-length experienced by an "average" bobolink during southward, post-nuptial migration (*cf.* Figure 3).

Group F

These three birds were exposed to 12¾-hour photoperiods (light intensity about 90 foot-candles) for six weeks; on November 13 the photoperiod was increased to 14 hours. Two birds developed beak pigmentation in early and mid-February, the last in the third week of March.

Group G

As in the previous group the initial photoperiods were 12¾ hours (light intensity 90 foot-candles), but these were continued for only five weeks; the 14-hour photoperiods were begun on November 6. One bird developed beak pigmentation surprisingly early, during the first week of December, another not until the third week of February. The third bird accidentally hung itself in the cage during the period December 22–26. At the time the accident was discovered the lower beak was discolored, but the testes were minute.

Group H

The initial photoperiods were 12½ hours (light intensity about 45 foot-candles) and they were continued for only four weeks. On October 30 the photoperiods were increased to 14 hours. One bird developed beak pigmentation during the last week in January, the other two in early February.

General conclusion

The post-nuptial photorefractoriness exhibited by bobolinks at the beginning of October can be terminated by only five weeks of relatively long, 12¾-hour, photo-

periods or by only four weeks of 12½-hour photoperiods. These lighting schedules approximate the day-lengths experienced by bobolinks in post-breeding migration.

DISCUSSION

Bartholomew (1949) pointed out that photorefractoriness might play an important role in regulating the timing of the annual recrudescence of the testes in *Passer domesticus*. It may be a significant factor in many passeriform birds in which the annual testicular cycle is controlled by photoperiodism (Wolfson, 1952; Wolfson, 1958). It assures that the gonad-stimulating mechanism does not again become activated, following regression and reconstitution of the inactive testes, until middle or late autumn when days are short, and continue to shorten, and the photoperiodic stimulus is therefore weak at best. Recrudescence of the testes, and the appearance of male sexual behavior, with ultimate development and release of motile spermatozoa, is thus suitably delayed.

In the absence of experimental evidence it was not easy to fit transequatorial migrants into this picture, because in post-nuptial migration they pass directly from the shortening days of the northern hemisphere autumn into the lengthening days of the southern hemisphere spring, never experiencing the retarding effect of the short days of winter. This consideration leads logically to the question of the photorefractory phase in such migrants. Bissonnette (1937) had suggested that "prolonged refractory periods . . . would supply the necessary delay to prevent even transequatorial migrants from breeding in their southern range" (p. 263). Farner (1954) postulated for transequatorial migrants "a characteristically longer refractory period" (p. 29). Wolfson (who since 1958 has preferred the term "preparatory phase") has spoken of the relation between day-length and the photorefractory phase as the "main problem" in equatorial and transequatorial migration and of the regulation of this phase as the "critical problem" (Wolfson, 1959, pp. 706–7; Wolfson, 1960, p. 785). Wolfson and Westerhoff (1960), in a report on some preliminary experiments with bobolinks, suggested that in this species, as compared with temperate zone species, a *longer period* of short days may be required in the regulation of the preparatory phase.

In all temperate zone species so far investigated, the refractory period is terminated in nature in middle to late autumn, that is, variously between mid-October and mid-November or even early December (published data summarized by Farner, 1954; Farner, 1959; Wolfson, 1958). It seems evident from our first series of experiments that, under comparable conditions (*i.e.*, the natural day-lengths of middle latitudes in the northern hemisphere), photorefractoriness in *Dolichonyx* may be terminated as early as November 1. Since normal reproductive activity in the preceding season had been suppressed in these captive birds, testicular regression may have been accelerated, leading possibly to an earlier termination of refractoriness. However, in some temperate zone species refractoriness persists until mid-November and in at least one species until early December (*Zonotrichia albicollis*, Lat. 42° N., Shank, 1959). Thus, even if we allow two to five weeks for a possible effect of the celibacy imposed on our captive birds, it would seem that *Dolichonyx* does not differ markedly from temperate zone migrants with respect to the timing of release from refractoriness in the northern hemisphere autumn.

The present experiments also permit comparison of *Dolichonyx* and temperate zone migrants in two other respects, namely, the length of the short days effective in terminating refractoriness, and also the number of such short days required. In previously reported experimental studies on the regulation of photorefractoriness by short days, photoperiods longer than 12 hours have not been employed (except by Wolfson and Westerhoff, 1960, for *Dolichonyx*). However, in at least some populations of *Junco hyemalis* and *Zonotrichia albicollis*, even eight weeks of exposure to 12-hour photoperiods, beginning October 1, does not release refractoriness (Engels, 1961). In another population of *Z. albicollis*, studied by Shank (1959), five weeks of 12-hour photoperiods, beginning October 1, failed to terminate refractoriness in any of nine males tested; thirty-one days of 9-hour photoperiods failed for five of eight males tested. In the present experiments, refractoriness in *Dolichonyx* was terminated by only four weeks of $12\frac{1}{2}$-hour photoperiods, and also by five weeks of $12\frac{3}{4}$-hour photoperiods, both beginning October 1. Therefore, the suggestion of Wolfson and Westerhoff, mentioned above, that *Dolichonyx* may require a longer period of short days to complete the "preparatory" phase, seems to be inapplicable. (Termination of refractoriness by November 1 under the influence of natural day-lengths at Lat. 36° N. also argues against this idea.) It may be suggested rather that in *Dolichonyx* one of the adjustments of the photoperiodic mechanism to transequatorial migration lies in the capacity to overcome refractoriness on relatively longer days, up to at least $12\frac{3}{4}$ hours.

Another adjustment, to the long days experienced between breeding seasons during the southern hemisphere summer, was indicated by earlier studies (Engels, 1961) which showed that, when bobolinks, juncos and white-throated sparrows were subjected to identical effective treatment for termination of refractoriness and stimulation of the gonad, testicular recrudescence in the bobolinks lagged several weeks behind recrudescence in the other two forms. It was suggested, at the time (p. 145), that this retardation of the bobolink cycle might be explained simply as evidence of a very slow rate of response (to 14-hour photoperiods) following the termination of refractoriness. An interesting alternative explanation might be that, during the period of exposure to shorter days, the photorefractory phase of *Dolichonyx* is not actually terminated (Engels, 1959, p. 764) but reaches a point where "longer days [no longer] prevent, but perhaps delay [its] completion" (Wolfson, 1960, p. 785). It was hoped initially that the present experiments would throw light on this question but, among other deficiencies, the number of birds used was too small to give the required information. It will be interesting to test the idea with experiments of a different design.

Examination of museum specimens indicates that the black pigmentation of the beak, which we used as a criterion of testicular recrudescence in bobolinks, in nature does not develop until April, when northward migration already is underway. Thus, in all of our experiments the development of this pigmentation was greatly accelerated, occurring in December, January, February or early March, even when we attempted to approximate, after October 1, the day-lengths expected to be encountered during the autumnal migration, followed by an approximation of the average day-length of the southern hemisphere summer. Obviously, much remains to be learned about the regulation of the natural timing of the testicular cycle in this transequatorial migrant.

SUMMARY

1. The testicular cycle of *Dolichonyx oryzivorus,* a bird which breeds above Lat. 40° N. and winters below Lat. 8° S., exhibits a photorefractoriness in early autumn, which is maintained by constant daily 14-hour photoperiods (experiment begun October 2).

2. Some individuals which had been held captive outdoors, exposed to the natural day-length of Lat. 36° N., were shifted to experimental, long photoperiods at various times between November 1 and November 28. Within a few months all of them developed the characteristic black beak pigmentation indicative of testicular recrudescence. From these results it is concluded that, under the influence of autumnal day-lengths of middle latitudes of the northern hemisphere, refractoriness is terminated by November 1 and that *Dolichonyx* thus does not differ appreciably from temperate zone species in the timing of this event under these conditions.

3. From published data on the time of autumnal migration within the United States and from data furnished by museum specimens collected south of the United States, an approximation of the cycle of day-lengths experienced by migrating bobolinks is constructed, which indicates that an "average" bobolink may experience in autumn only a few weeks of day-lengths less than about $12\frac{3}{4}$ hours.

4. Beginning October 2, when captive bobolinks were experiencing natural day-lengths of about 12 hours 41 minutes, some were exposed to constant daily photoperiods of $12\frac{1}{2}$ hours for four weeks, others to $12\frac{3}{4}$-hour photoperiods for five or six weeks, after which the photoperiod was increased to 14 hours. Testicular recrudescence occurred in all. It is concluded that photorefractoriness can be overcome in this species by only a few weeks of exposure to photoperiods which in length are comparable to those it encounters in transequatorial migration but which are longer than those which maintain refractoriness in such temperate zone forms as *Junco hyemalis* and *Zonotrichia albicollis.* The number of shorter days required for the termination of refractoriness seems to be of the same general order of magnitude as for *Junco* and *Zonotrichia.*

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